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The Effect of Slash Burning on the Vesicular-Arbuscular Mycorrhizal Inoculum Potential of Pinyon-Juniper Woodland Soils

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Introduction

Pinyon-juniper woodlands are the third largest plant community in the United States, occupying over 7 million hectares of the Southwest (Miller and Wigand 1994). There is concern that these already extensive woodlands are expanding at an unprecedented rate as a consequence of overgrazing, fire suppression and recent increases in annual precipitation (Miller et al. 1994), leading to ecosystem shifts from grass/shrub-dominated ecosystems to tree-dominated systems with little understory development. As a result, much of pinyon-juniper management has focused on improving understory plant establishment. Management towards this goal can involve fuel wood harvesting followed by burning of the remaining slash (Overby et al., in review).

The role of fire as a management tool has been debated because of the disturbance it creates in plant and microbial communities and in ecosystem processes (Campbell et al. 1977, Everett 1987, Walstad et al. 1990). Wildfires have the potential to eliminate aboveground plant communities (Campbell et al. 1977) as well as destroy belowground seed banks, fine roots and stores of fungal propagules (Swezy and Agee 1991, Wright 1996). Less intense controlled burns do not achieve the high temperatures associated with wildfire and thus, do not tend to eliminate all of the aboveground biomass (Martin 1978). However, less intense burns have the potential to smolder for long periods of time, which can be highly detrimental to belowground organisms, such as mycorrhizal fungi (Wright 1996).

The majority of plants present in pinyon-juniper communities are colonized by vesicular-arbuscular mycorrhizal (VAM) fungi (Klopatek et al. 1988), which play an

important role in plant nutrient and water uptake and soil stability (Smith and Read 1997). However, these fungi are sensitive to many of the disturbances prevalent in pinyon-juniper woodlands including grazing, soil compaction and fire (e.g. Bethlenfalvay and Dakessian 1984, Allen 1990, Klopatek et al. 1988). For example, the inoculum potential of forest soils for both ectomycorrhizal fungi (Buchholz and Gallagher 1982) and VAM fungi (Klopatek et al. 1988) can be reduced following fire. These reductions can be substantial, exceeding 95% in some soil types in pinyon-juniper woodlands (Klopatek et al. 1988). Given that many plant species are obligately dependent on associations with mycorrhizal fungi for establishment and growth (Smith and Read 1997), reductions in mycorrhizal inoculum potential can have important impacts on seedling establishment following fire.

The primary goal of this study was to examine the effects of fire on the VAM inoculum potential of soils in a pinyon-juniper woodland, where fuel wood was harvested and the slash burned in 1995. Understory plant production, particularly for native perennials was poor following treatment at this site (W. Moir, pers. comm., Overby et al., in review). In addition, the abundance of exotic plant species was frequently greater on the burned sites than on nearby non-burned sites (W. Moir, pers. comm.). Many exotic species are either non-mycotrophic or facultatively mycotrophic and are thus able to establish and survive without associations with mycorrhizal fungi (Allen 1990). Therefore, we proposed to test the following hypothesis: The burning of pinyon and juniper slash causes persistent reductions in the VAM inoculum potential of pinyon-juniper woodland soils, thereby resulting in reductions in the VAM colonization of VAM-dependent understory plants. We also predict that plant growth rates will be lower

in the burned sites due to the reduced mycorrhizal colonization of plants grown in these soils. Our study differs from previous research in that we are examining whether mycorrhizal inoculum remains limited in soils five years after burning rather than looking at the shorter-term responses addressed in other studies.

Study Site

This study was conducted at Hogg Pasture, a pinyon-juniper woodland, located approximately 40 miles south of Flagstaff, Coconino County, AZ off of Interstate 17. Pinyon pine (*Pinus edulis*) and Utah juniper (*Juniperus osteosperma*) are the co-dominant plants at this site. The soils of this study area have been classified as Typic Haplustalf, fine, smectic, mesic (Overby et al. in review). This area was opened up to fuel wood harvesting and the resulting slash was burned in 1995.

Methods

VAM bioassay: We tested our hypothesis by performing a bioassay of the mycorrhizal inoculum potential of soils from four site types: 1) burned soils from beneath the canopy of burned trees (as determined by the presence of a burned stump), 2) burned soils from the interspace between burned canopies, 3) non-burned soils from beneath the canopy of either juniper or pinyon, and 4) non-burned soils from interspace areas. The non-burned soils were collected from nearby control sites of the same soil and vegetation type that were not burned in 1995. A total of 16 transects were established in both burned sites (n=8) and non-burned, control sites (n=8). Five soil cores from canopy areas and five soil cores from interspace areas were collected to a

depth of 10 cm along each transect in April 2000. Soil cores were transferred in the field to Conetainers™ and then returned to the greenhouse.

Cores were sown with a single, surface-sterilized (10% bleach for 30 minutes), organic corn (*Zea mays*) seed. The seeds were covered with 2.5 cm of Vermiculite to help retain soil moisture. The pots were kept at the Forest Service greenhouse off of Lone Tree Road, Flagstaff, AZ. Average temperatures in the greenhouse were maintained at 25° C (day) and 15° C (night) with a 16-hour light and 8-hour dark light regime. Seeds were watered daily until germination at which time they were watered every other day. The majority of seeds germinated within 7 days. Seeds that did not germinate after one week were removed and replaced with a fresh surface-sterilized seed. The necessary additional growing time was calculated into the harvest date. Plants were allowed to grow for 5 weeks from their date of germination, at which time they were sacrificed. The corn plants were grown for only five weeks because previous research has shown that the level of root colonization by mycorrhizal fungi is linearly related to soil densities of VAM fungal propagules early in the colonization process (e.g., Smith and Walker 1981). Both wet and oven dry shoot and root weights were obtained.

VAM colonization: A small randomly chosen portion of corn root was selected from each plant for root clearing and staining (Philips and Hayman 1970). Briefly, this method involved clearing root samples in 10% potassium hydroxide, bleaching them in 3% hydrogen peroxide, and staining them in 0.5% trypan blue in lactoglycerol. Vesicular-arbuscular mycorrhizal colonization was determined by mounting a stained root specimen on a microscope slide and viewing it under a compound microscope at

400X magnification. Counts were made using the point intersect method (McGonigle et al. 1990). Roots were considered to be colonized if the reticle line of the compound microscope intersected an arbuscule, a vesicle, or an internal hypha.

Statistical analyses: We used a two-way ANOVA to test for differences in VAM colonization, plant biomass and shoot/root ratio, with burning regime (burned vs. control) and canopy presence (canopy vs. interspace) as fixed factors (SPSS 10.0 software). If differences were detected in the two-way ANOVA, then post-hoc Tukey's tests were performed to determine treatment differences. Because the VAM data are proportional, they were subjected to arcsin-square root transformations prior to analysis. Data for VAM presented in this report represent 13 out of 40 samples while plant biomass and shoot/root ratio data were determined from 36 samples or more.

Results

Contrary to our hypothesis, VAM colonization at the burned and non-burned sites was similar indicating that levels of soil inoculum were comparable at the two site types. There was also no significant difference in VAM colonization between the canopy and interspace samples, nor was there a significant interaction between burning and canopy location **(Table 1, Figure 1)**. However, these analyses are based on 13 of the 40 samples. Analysis of the remainder of the samples will be provided at a future date (levels of colonization are still being determined on some of the samples). There was a trend for plants grown in soils from the burned areas to have greater colonization, however this pattern was blurred by the high variance.

Corn plants that were grown in soil from the burned-canopy sites had significantly greater total biomass than plants grown in soil from the control sites **(Table 1, Figure 2a:** Tukey results, $p<0.001$**)**. However, there was no significant difference in the biomass of plants grown in soils from beneath the tree canopy or from interspaces, nor was there a significant burn by canopy interaction **(Table 1, Figure 2a)**. There are two hypotheses that may explain this result. First, the slight difference we observed in the burned vs. control plots in mycorrhizal colonization may be sufficient to affect plant growth. Second, and more likely, there may be more nutrients available to the plants in the burned soils, due to post-burning nutrient release.

Neither burning nor canopy location had a significant effect on the shoot/root ratios of the corn seedlings, nor was there a significant burn by canopy interaction **(Table 1, Figure 2b)**. This result suggests that energy allocation to shoots and roots was similar across all treatment groups.

Discussion

Our results suggest that prescribed fire does not lead to persistent differences in the mycorrhizal inoculum potential of pinyon-juniper woodland soil. Five years following burning, the mycorrhizal inoculum potential of burned soils was slightly but not significantly higher than the mycorrhizal inoculum potential of non-burned soils. Our results contrast with those of Klopatek et al. (1988) who observed dramatic reductions in mycorrhizal inoculum potential immediately following a simulated fire in experimental soil microcosms. There are two possible explanations for our findings relative to previous research. First, our sample size is currently small ($n = 13$) and may be

insufficient to detect differences among the treatment groups. Second, our study was conducted five years after piles of slash were burned. During the years following burning, inoculum potentials may have been restored, either by wind blown or animal-dispersed spores or via inoculated roots growing in from surrounding non-burned areas. Disturbed sites can be quickly recolonized by VAM fungi in the presence of animal vectors and prevailing winds (Allen 1990).

We also observed no significant differences between canopy and interspace microhabitats in mycorrhizal inoculum potential regardless of burn treatment. Klopatek et al. (1988) also observed no significant differences among canopy and interspace locations in non-burned soils, but found that interspace soils had higher levels of mycorrhizal inoculum potential than canopy soils following burning, particularly if the burn was a wet burn in which microcosms were wetted to 50% field capacity prior to burning. This difference may also be explained by the extensive time period following burning in our study and the ability of even the severely burned canopy sites to become recolonized with mycorrhizal propagules following the fire.

The greater growth rates of corn seedlings in the burned soils than in the non-burned soils suggests that conditions for plant growth are not persistently reduced following the burning of slash. Although corn growth rates may not be indicative of the growth rates of native plants, our findings still suggest that conditions for non-woody plant growth are not seriously compromised five years following burning. In fact, the opposite was true with corn achieving the highest growth rates in the burned canopy sites. Interestingly, Overby et al. (in review) observed that organic carbon and available ammonium, nitrate, and phosphate were significantly higher in the burned canopy sites

than in the other three sites two years following burning. If these differences persisted, they could provide an explanation for the higher levels of growth in the burned canopy plots.

Our combined findings of equivalent levels of mycorrhizal inoculum potential and increased corn growth rates at the burned sites suggest that mechanisms other than the abundance of mycorrhizal propagules have a greater influence on the establishment of native vegetation following slash burning. For example, the fire may have had a more persistent effect on the seed bank of native perennial plant species, while the seed banks of native annuals and exotics were less compromised.

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Table 1. Results from two-way ANOVA's for VAM colonization, plant biomass and shoot/root ratio are presented. Fixed factors were burning regime (burned vs. control) and canopy presence (canopy vs. interspace). Asterisks indicate statistical significance at α=0.05 level.

Factor	VAM colonization	Biomass	Shoot/Root Ratio
Burning regime	$F_{1,48}$=1.012, p=0.32	$F_{1,152}$=18.08, **p=0.00***	$F_{1,152}$=0.03, p=0.86
Canopy presence	$F_{1,48}$=0.089, p=0.77	$F_{1,152}$=2.33, p=0.13	$F_{1,152}$=0.02, p=0.97
Interaction	$F_{1,48}$=1.189, p=0.28	$F_{1,152}$=1.38, p=0.24	$F_{1,152}$=3.36, p=0.07

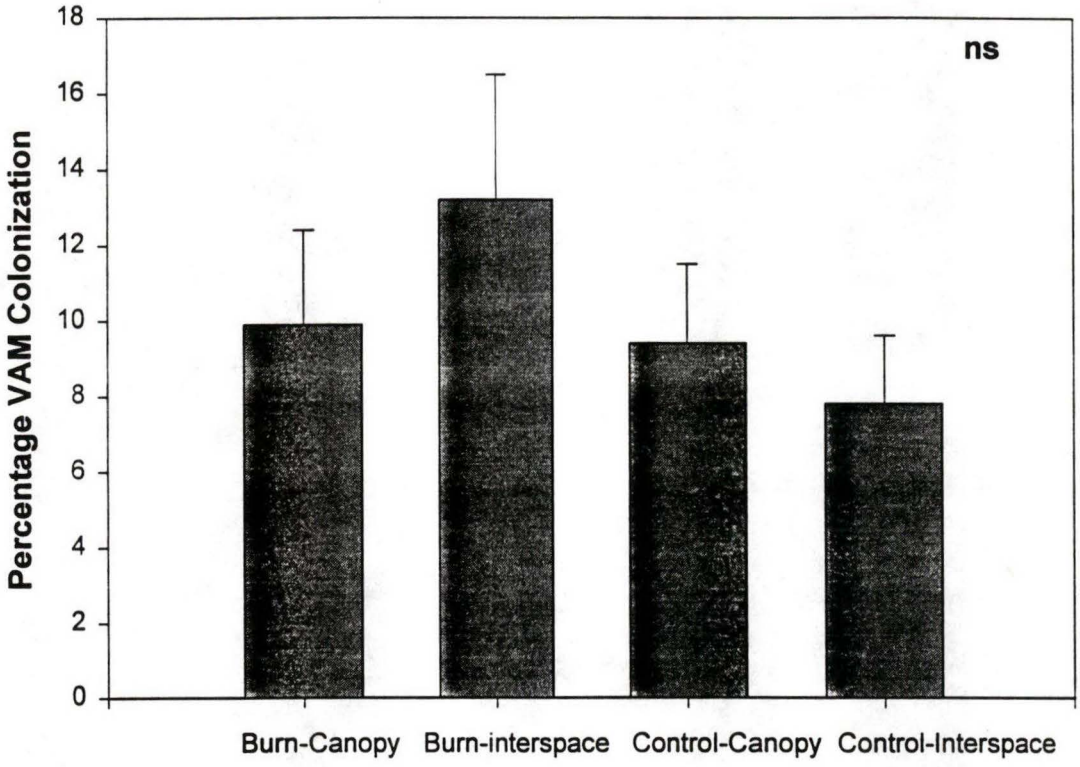

Figure 1. Means and standard errors are presented for percentage VAM colonization. There were no significant differences among the treatments (n=13).

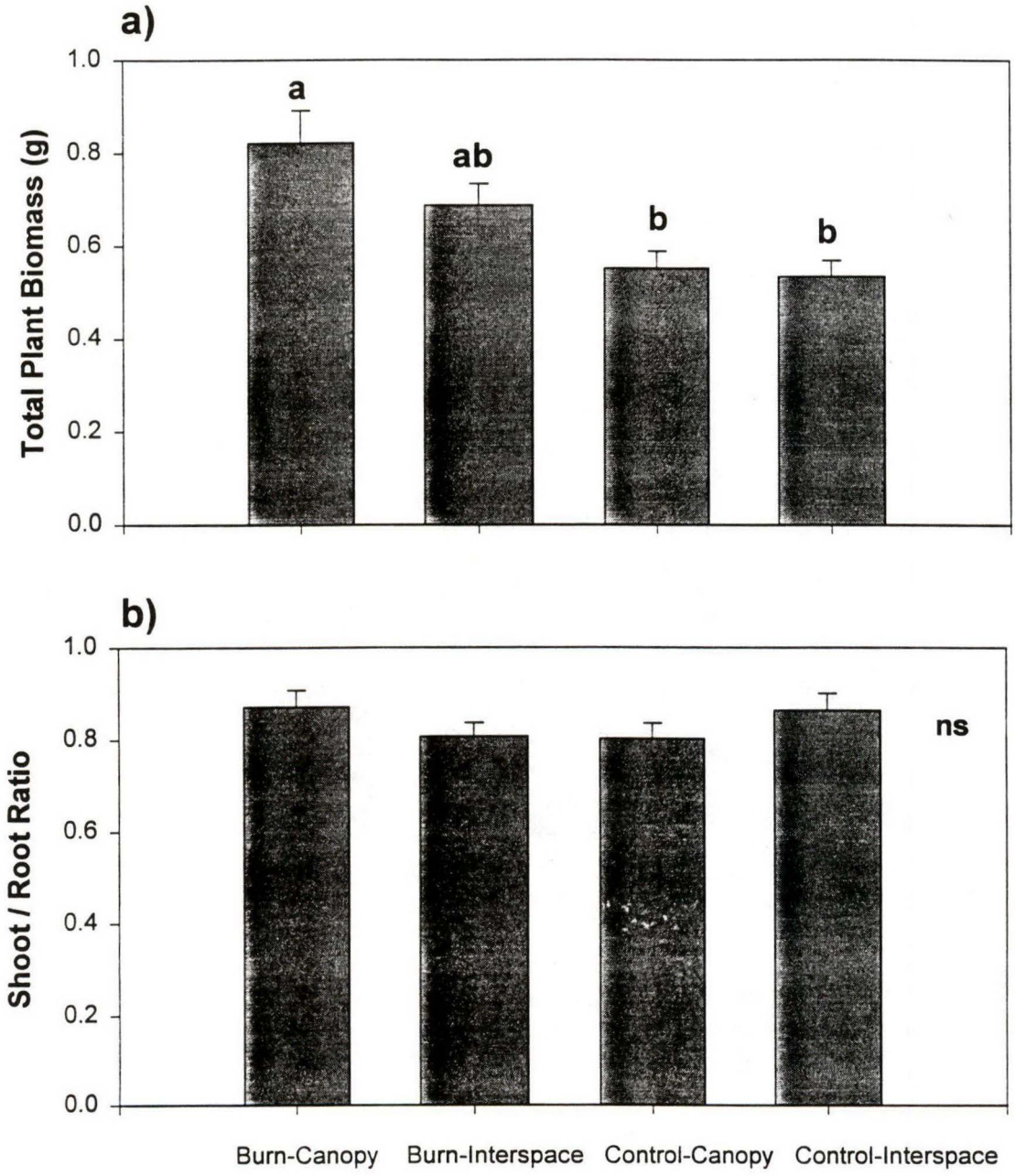

Figure 2. Means and standard errors are presented for total plant biomass (a) and shoot/root ratio (b). Columns with different letters are significantly different from one another at the 0.05 level.